

Claims

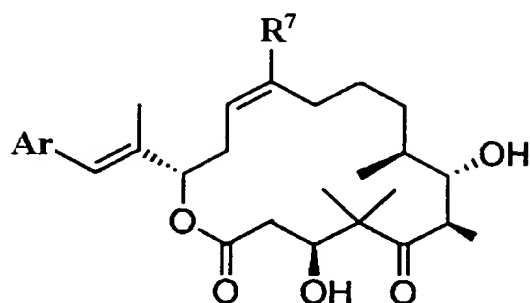
1. A recombinant host cell of the suborder *Cystobacterineae* containing a recombinant expression vector that encodes a heterologous polyketide synthase (PKS) gene and produces a polyketide synthesized by a PKS enzyme encoded on said vector.
2. The host cell of Claim 1 that is selected from the family *Myxococcaceae*.
3. The host cell of Claim 1 that is selected from the family *Cystobacteraceae*.
4. The host cell of Claim 2 that is selected from a genus selected from the group consisting of *Angiococcus*, *Myxococcus*, and *Corallococcus*.
5. The host cell of Claim 3 that is selected from a genus selected from the group consisting of *Cystobacter*, *Melittangium*, *Stigmatella*, and *Archangium*.
6. The host cell of Claim 4 that is selected from the genus *Myxococcus*.
7. The host cell of Claim 5 that is selected from the genus *Stigmatella*.
8. The host cell of Claim 6 that is selected from the group consisting of *M. stipitatus*, *M. fulvus*, *M. xanthus*, and *M. virescens*.
9. The host cell of Claim 7 that is selected from the group consisting of *S. erecta*, and *S. aurantiaca*.
10. The host cell of Claim 8 that is *Myxococcus xanthus*.
11. A method for producing a polyketide in a host cell of the suborder *Cystobacterineae*, which polyketide is not naturally produced in said host cell, said

method comprising culturing a host cell of Claim 1 under conditions such that a PKS gene encoded on the vector is expressed and said polyketide is produced.

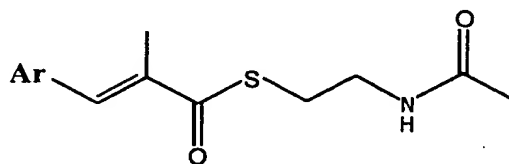
12. The recombinant host cell of Claim 1 that produces epothilone or an epothilone derivative.
13. The host cell of Claim 10 that produces epothilone or an epothilone derivative.
14. The host cell of Claim 13 that produces epothilones A, B, C, and D.
15. The host cell of Claim 14 that is *Myxococcus xanthus* K111-32.25.
16. The host cell of Claim 14 that produces epothilones A and B as major products and epothilones C and D as minor products.
17. The host cell of Claim 13 that produces epothilones C and D as major products.
18. The host cell of Claim 17 that either does not contain an *epoK* gene or does not express a fully functional *epoK* gene product.
19. The host cell of Claim 18 that is *Myxococcus xanthus* K111-40.1.
20. The host cell of Claim 18 that is *Myxococcus xanthus* K111-72.4.4.
21. The host cell of Claim 13 that contains an epothilone PKS gene in which a coding sequence for a module of said PKS has been altered by mutation, deletion, or replacement.
22. The host cell of Claim 21, wherein said module is extender module 6.

23. The host cell of Claim 22, wherein said module lacks a functional ketoreductase domain and produces a 9-keto epothilone.
24. The host cell of Claim 21, wherein said module is extender module 5.
25. The host cell of Claim 24, wherein said module 5 lacks a functional dehydratase domain and produces a 13-hydroxy epothilone.
26. The host cell of Claim 21, wherein said module is extender module 4.
27. The host cell of Claim 26, wherein said module lacks a functional ketoreductase domain and produces a 13-keto epothilone.
28. The host cell of Claim 21, wherein said module is extender module 2, the coding sequence for the ketosynthase domain has been altered by mutation to change an active site cysteine to another amino acid, and which host cell must be provided a diketide equivalent compound to produce an epothilone or epothilone derivative.
29. The host cell of Claim 28 that is *Myxococcus xanthus* strain K90-132.1.1.2.
30. The host cell of Claim 21, wherein said module is extender module 1.
31. The host cell of Claim 30, wherein said module has been changed so that it binds an amino acid other than cysteine.
32. The host cell of Claim 21, wherein said module is a loading module.
33. The host cell of Claim 32, wherein said module has been replaced with a module that binds an amino acid.

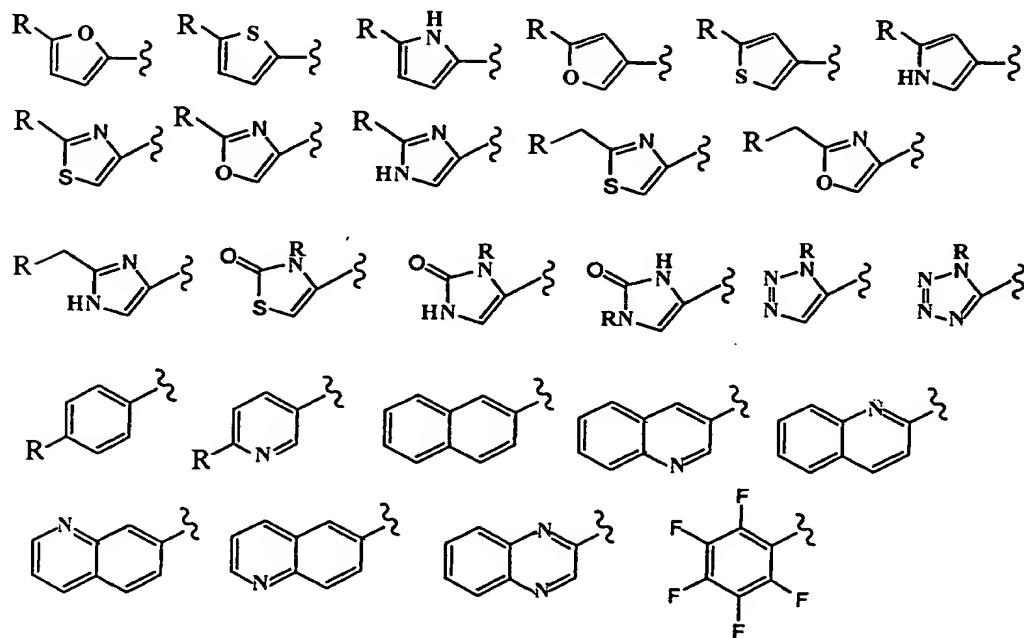
34. An epothilone derivative of the formula



produced by culturing said host cell of Claim 28 with a diketide equivalent compound of the formula



where R^7 is hydrogen or methyl and Ar is aryl is selected from the group consisting of



where R is hydrogen, hydroxy, halogen, amino, C_1 - C_5 alkyl, C_1 - C_5 hydroxyalkyl, C_1 - C_5 alkoxy, and C_1 - C_5 aminoalkyl.

35. The host cell of Claim 1 that further comprises a heterologous gene that encodes for an enzyme selected from the group consisting of an enzyme that transports a compound into said cell that is utilized in biosynthesis of the polyketide, an enzyme that synthesizes a compound utilized in biosynthesis of the polyketide, and an enzyme that phosphopantetheinylates a PKS.

36. The host cell of Claim 35, wherein said enzyme is MatB.

37. The host cell of Claim 35, wherein said enzyme is MatC.

38. The host cell of Claim 35, wherein said enzyme is MtaA.

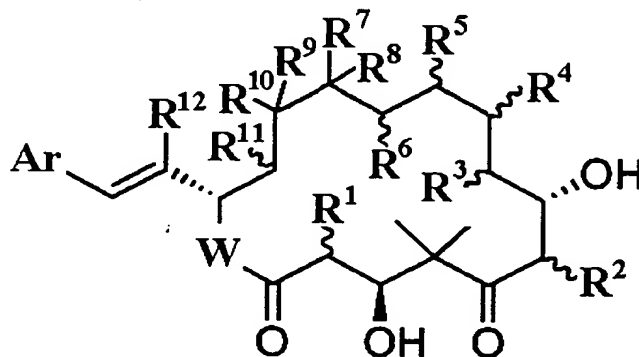
39. The host cell of Claim 13, wherein said epothilone or epothilone derivative is produced by a PKS gene under the control of a promoter selected from the group consisting of a promoter from an *S. cellulosum* epothilone PKS gene, a promoter from a myxothiazol biosynthesis gene, a promoter from a TA biosynthesis gene, a *pilA* promoter, a promoter from a kanamycin resistance conferring gene, and a So ce90 promoter.

40. A method for purifying an epothilone from a cell that produces epothilone, said method comprising culturing said cell in the presence of XAD resin, eluting epothilone from said resin, performing a solid phase extraction of epothilone eluted from said resin, and performing chromatography on epothilone resulting from said solid phase extraction.

41. The method of Claim 36 that further comprises a crystallization step.

42. Crystalline epothilone D.

43. A method for fermentation of a *Myxococcus* host cell, which method comprises culturing said cell in liquid medium comprising a fatty acid or oil as a carbon source.
44. The method of Claim 43, wherein said fermentation is a fed-batch fermentation.
45. The method of Claim 43, wherein said *Myxococcus* host cell produces an epothilone or an epothilone derivative.
46. The method of Claim 45, wherein said host cell produces an epothilone derivative that contains an oxazole instead of a thiazole, and said liquid medium comprises L-serine.
47. A isolated compound of the formula



wherein:

R¹, R², R³, R⁵, R¹¹, and R¹² are each independently hydrogen, methyl or ethyl;

R⁴, R⁶ and R⁹ are each independently hydrogen, hydroxyl, or oxo;
alternatively

R⁵ and R⁶ together form a carbon carbon double bond;

R⁷ is hydrogen, methyl, or ethyl;

R⁸ and R¹⁰ are both hydrogen or together form a carbon carbon double bond or an epoxide;

Ar is aryl; and,

W is O or NR^{13} where R^{13} is hydrogen, $\text{C}_1\text{-C}_{10}$ aliphatic, aryl or alkylaryl.

106160"EB429660